

Claims

We claim

1. A method for the rescue of attenuated and/or recombinant orthomyxovirus, comprising preparing a recombinant baculovirus by inserting at least one natural
5 or modified gene or gene segment or a combination of a least one natural and at least one modified gene or gene segment, of orthomyxovirus into the genome of a baculovirus, delivering the resulting recombinant baculovirus genome to a mammalian host cell by transduction and incubating the transduced host cell to allow for expression of said one or more orthomyxovirus genes or gene segments.
- 10 2. The method of claim 1, wherein the host cell is a VERO cell.
3. The method of claim 1 wherein all genes or gene segments of the attenuated and/or recombinant orthomyxovirus are transduced into the host cell by means of recombinant baculovirus.
4. The method of claim 3, wherein all of said genes or gene segments are inserted
15 into a single baculovirus genome and are transduced all together into the host cell.
5. The method of claim 3, wherein said genes or gene segments are divided into portions and distributed to two or more baculovirus genomes and subsequently delivered to the host cell by co-transduction.
- 20 6. The method of claim 1, comprising delivery of at least one of said natural or modified orthomyxovirus genes or gene segments to the host cell by baculovirus transduction, and subsequent infection of the transduced host cell with a helper virus followed by selection for modified, i.e. reassorted, helper virus wherein said at least one transduced gene or gene segment replaces the corresponding gene or
25 gene segment of the helper virus.
7. The method of claim 1, wherein said at least one modified gene or gene segment confers attenuation upon the rescued virus.
8. The method of claim 1, wherein the modified gene segment is a modified NS gene segment of influenza virus encoding an NS1 protein of only 38 amino acids
30 in length (Δ NS38).
9. The method of claim 1, wherein said at least one natural or modified gene or gene segment is flanked by restriction sites and wherein introduction and exchange of said gene or gene segment is carried out by cleavage of said restriction sites and

subsequent direct ligation of said at least one gene or gene segment into the baculovirus genome.

10. Baculovirus genome that contains one or more uni- or bidirectional expression cassettes encoding at least one natural or modified gene or gene segment or a combination of a least one natural and at least one modified gene or gene segment, of an orthomyxovirus.
11. Baculovirus genome according to claim 10, wherein said at least one natural or modified gene or gene segment is from influenza A or B virus.
12. Baculovirus genome according to claim 10, wherein said at least one natural or modified gene or gene segment encodes viral mRNA or viral genomic RNA, or both viral mRNA and viral genomic RNA.
13. Baculovirus genome according to claim 10, wherein said at least one natural or modified gene or gene segment is flanked by restriction sites that allow for introduction and exchange of said gene or gene segment by cleavage of said restriction sites and subsequent direct ligation into the baculovirus genome.
14. Baculovirus genome according to claim 10, wherein said at least one natural or modified gene or gene segment comprises a modified NS gene segment of influenza virus encoding an NS1 protein of only 38 amino acids in length (Δ NS38).
15. Baculovirus genome according to claim 10, wherein said at least one natural or modified gene or gene segment comprises a modified NS gene segment of influenza virus, a hemagglutinin (HA) and/or a neuraminidase (NA) gene segment of an epidemic influenza strain, and all remaining gene segments of said epidemic strain or of one or more other influenza wildtype or laboratory master strains.
16. Recombinant baculovirus comprising a modified genome as defined in claim 10.
17. A method for the manufacture of an orthomyxovirus vaccine, comprising transducing a baculovirus genome as defined in claim 10 into a mammalian host cell, incubating the host cell to allow for expression of the transduced viral genes and for assembly of mature orthomyxovirus particles, separating and purifying the orthomyxovirus particles and combining them with a suitable carrier.
18. The method of claim 17, further comprising infecting the host cell with a helper virus after transduction of the baculovirus genome into the host cell.

19. The method of claim 17, further comprising processing the purified orthomyxovirus particles to obtain infectious virions.
20. The method of claim 17, for the manufacture of a live attenuated influenza vaccine.